Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 7, 30, 31, 33 and 34 stand rejected under 35 U.S.C. §112, first paragraph as allegedly failing to provide an enabling disclosure. In summary, the rejection states that success in obtaining ES cells in mammals other than murine using the disclosed methods are unpredictable. The Examiner cites Notarianni, et al. and Bradley, et al. to support his position.

Applicant respectfully points out that substantially the same rejection under 35 U.S.C. §112, first paragraph was set forth in the July 15, 1994 Office Action in the parent application U.S. Serial No. 07/958,562. The rejection in the parent application used Evans et al. (AB) to support the allegation of lack of enablement. This rejection was overcome by applicants arguments and declarations set forth therein.

As successfully noted in the parent application, applicant respectfully submits that the Examiner's position is not scientifically supported. Notariunni et al., Bradley et al. and Evans et al. all concern a very different procedure which cannot be extrapolated to the subject application.

Specifically, Notarianni et al., Bradley et al. and Evans et al. were concerned with a method of deriving embryonic stem cells from a blastocyst stage embryo. A blastocyst is a

very early embryo which has not yet implanted onto the uterine wall. At the time of the applicants invention it was known that other labs had tried very hard to isolate pluripotent embryonic stem cells from rat, pig and sheep without success. What seems to happen is that the inner cell mass cells differentiate very easily into endoderm and they fail to proliferate further as pluripotent embryonic stem cells. (In fact, there have been reports within the last year of success in obtaining cells from Rhesus monkey blastocysts and perhaps one recent success from rat blastocysts.) In view of the perceived problems isolating pluripotent stem cells from blastocysts and the differences between early embryos in divergent species, one skilled in the art would not expect that methods of obtaining stem cells from mice blastocysts would be directly applicable to other species.

Importantly, applicant's claimed invention does not use blastocysts to derive embryonic stem cells. Applicant uses a very different method to derive embryonic stem cells. As disclosed throughout the application and the murine and human primordial germ cell Examples, applicant's embryonic stem cells are derived from primordial germ cells dissected from post-implantation embryos.

Importantly, post-implantation embryos are very consistent between species as to the location of the primordial germ cells. The site at which primordial germ cells (PGCs) can

be first detected (at the base of the allantois and yolk sac), their migration along the hind gut and their entry into the embryonic gonads is, based on the literature, highly conserved in mammalian embryos (see, for example, Hamilton and Mossman, *Human Embryology* 4th edition, pp. 401-403, The Macmillan Press Ltd., London (1972) (Exhibit A) and Moore and Persaud, *The Developing Human: Clinically Oriented Embryology* 5th edition, p. 281-283, W. B. Saunders Co., Philadelphia (Exhibit B), for human; Hendrickx and Sawyer, "Embryology of the Rhesus Monkey," *The Rhesus Monkey* Vol. II, Ed. G.H. Bourne, pp. 141-169, Academic Press, New York (1993)(Exhibit C), for Rhesus Monkey; and Schoenwolf, *Laboratory Studies of Vertebrate and Invertebrate Embryos*, 7th Edition, Prentis Hall, New Jersey (1995) (Exhibit D), for porcine). This means that it is possible to predict with a high degree of confidence where to find primordial germ cells in mammalian embryos of different species at a given state of embryonic development. Thus, one would reasonably expect success in different species using the claimed methods.

In view of these distinctions, it is clear that the concerns of Notarianni et al., Bradley et al. and Evans et al. simply do not apply to the claimed invention. Thus, the Examiner has not adequately supported the unpredictability by adequate art and the rejection under 35 U.S.C. § 112 should be withdrawn.

In the parent application, applicant had attached hereto a Declaration by Dr. Brigid L. M. Hogan (Exhibit E) setting forth data showing the isolation and proliferation of primordial germ cells from a post-implantation human embryo. The methods set forth in the Declaration to isolate human primordial germ cells are the same methods utilized for mice in the subject application with routine modifications based on the different age of the mouse and human embryos. This data was incorporated into the subject application and provides further evidence of the predictability of the method.

In summary, because 1) the prior art statements are not applicable to the post-implantation embryos used in the methods to derive the claimed cells or 2) the human primordial cell data shows that applicant's method can be routinely applied to primordial germ cells of different species, the rejection under 35 U.S.C. § 112, first paragraph should be withdrawn. Again, this evidence was sufficient to overcome a very similar rejection in the parent application.

However, to even mere clearly set forth the predictability of applicants claimed methods applicants now have data demonstrating the isolation of two human ES cell lines. (Applicants note that ES cells derived from primordial germ cells are also called EG cells in the literature following applicants invention).

Specifically, fetal material from pregnancy terminations was collected on a weekly basis. The general procedures set forth in the subject application and claimed in U.S. Patent No. 5,453,357 were applied to this human fetal material. Two preliminary attempts were used to establish dissection procedures and cell culture conditions, and failed to result in EG cultures. The third attempt resulted in two separate cell cultures, termed hEG8-1 and hEG9-1. These cells were passaged 4 times to expand cell numbers, and multiple frozen stocks from each passage were saved. Most importantly, the cells demonstrated a tightly clustered and rapidly growing morphology reminiscent of early passage mouse ES and EG cultures (see Exhibit F, Figure 1a-d), and demonstrated strong and convincing histological staining for alkaline phosphatase (see Exhibit F, Figure 2a-d).

Therefore, the application as filed enables one skilled in the art to apply this novel method of deriving ES cells to all mammals of interest and very clearly enables one to derive human ES cell lines.

The Examiner also alleges that there is no enabling disclosure for Claims 33 and 34 because there is no guidance how one would prepare therapeutic ES cell derivatives or how they would be determined to be therapeutic. However, the Examiner, on

page 6 of the Office Action, states that "it is noted that ES cells that have been modified and used to prepare animals have been derivatized and evaluated for their ability to alter the phenotype of animals. Thus, by this use it would have been determined as to whether such cells would have been useful for therapies such as correcting or modulating genetic mutations." In view of this statement it is clear that Claims 33 and 34 are enabled when considered in view of the art cited by the Examiner.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claim 2 is alleged to be indefinite as to the meaning of "non-functional" and Claim 3 is alleged to be indefinite in the meaning of "functional".

Applicants respectfully traverse this rejection. Page 10, lines 1-7 discuss the concept of functionality. Specifically, functional genes are genes which can produce their gene product and non-functional genes cannot produce their gene product. Such a meaning would be clear to one skilled in the art. In this regard, applicant notes that the Examiner on Page 6, lines 3-22, seems to understand these meanings and states that, indeed, the art provides examples of a functional neo gene. In view of these factors applicants respectfully requests that this rejection be withdrawn.

Claims 5-7 are stated to be unclear in the Office Action. Claims 5 and 6 have been canceled above. As regards Claim 7, applicant notes that this language was deemed to be clear in U.S. Patent No. 5,453,357. To clarify, the limitation applies to all the factors. Further, to enhance the growth means that growth is better than if the factor was not added.

Claims 33 and 34 have been amended above to include an obtainment step. The rejection on this basis should be removed.

Rejection Under 35 U.S.C. §102(b)

The Examiner alleges that Evans et al. anticipates Claim 1 because Evans et al. discloses the production of bovine and porcine pluripatential embryonic stem cells. However, as noted by the Examiner in the December 4, 1995 Office Action, Bradley et al. in May 1992 states:

> A number of reports have claimed the isolation of ES cells from farm animals such as pigs and sheep. However, the description of these cell lines is yet to be supported by the demonstration that they can proliferate and differentiate in an embryo in vivo, contributing somatic tissues or germ cells.

This quote in Bradley cites Notarianni et al. The data in Notarianni et al. also is presented in Evans et al. Thus, applicants respectfully assert that Evans et al. have not obtained ES cells from bovine and porcine as noted by Bradley et al. In further support of this notion, Evans et al. has a 1989 international filing date yet no patent has issued or further confirmation of success in the literature has occurred.

Rejection Under 35 U.S.C. §102(a)

Claims 5 and 6 stand rejected under 35 U.S.C. §102(a) over Matsui et al. Attached hereto is a copy of a signed Declaration by Dr. Brigid L. M. Hogan (Exhibit G) stating that Yasuhisa Matsui and Krisztina Zsebo did not contribute to the conception of the claimed invention. This copy was submitted in the parent application to overcome this same rejection. Specifically, Yasuhisa Matsui was a post doctoral student from Japan and only acted as Dr. Hogan's direction and supervision. Krisztina Zsebo only provided the steel factor at Dr. Hogan's request.

Therefore, the inventive entity is the same for Matsui et al. and the subject application and the rejection under 35 U.S.C. §102(a) should be removed.

Rejection Under 35 U.S.C. §103

Claims 2, 3, 30 and 33 stand rejected under 35 U.S.C. §103 as allegedly unpatentable over Evans et al. in view of McMahon et al. Claim 31 stands rejected under 35 U.S.C. §103 as allegedly unpatentable over Evans, et al. in view of Pratt.

As noted above, based on the literature Evans et al. did not have the ES cells they claimed. Thus, the primary reference is deficient and the secondary references do not cure this deficiency. Therefore, these rejections under 35 U.S.C. §103 should also be removed.

Applicants note that Claims 4, 7 and 34 are free of the prior art. In view of the enablement set forth above, applicants respectfully note that these claims are now in condition for allowance.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A check in the amount of $450.00 is attached as required for the extension of time. This amount is believed to be correct; however, the Commissioner is hereby authorized to

charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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I hereby certify that this **AMENDMENT** is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

on this 4 day of June, 1996.

David G. Perryman 6-4-96
DATE

Respectfully submitted,

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